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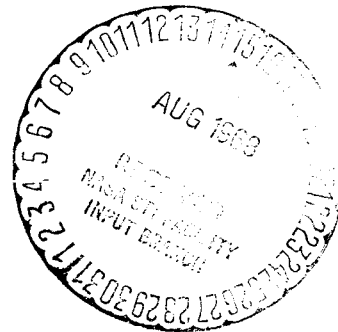
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EXPERIMENTAL INVESTIGATION OF SEX TRANSFORMATION IN THE FROG

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ABSTRACT: Presents results on late fertilization of eggs of *Rana fusca* and the effect of this on the sex ratio.

From the results of investigations carried out by Pflüger, R. Hertwig and their students, we know that the sex of young froglets is very unstable. Not only can the ratio of males to females be shifted by certain manipulations, but deviations from the normal ratio may also be observed in the field, and this in particular among young animals. In older animals, a normal ratio is achieved by a transformation of individuals of the excess sex into those of the other one. Until now apparently only transformation of females into males has been observed in the frog (Witschi 1921). A transformation in the opposite direction is said to be not possible for a variety of theoretical reasons. I believe that the results of my investigations, which originally were designed to study a set of entirely different questions, ** demonstrate the occurrence of transformation of males into females.

/386*

Materials

In the experimentation concerning the numerical sex ratio in the frog, one must be well acquainted with the characteristics of the strains employed. Here, at Dorpat (Tartu), *Rana fusca* (Rö.s.) spawns relatively late in the season, i. e., between the middle and the end of April. The ratios of females to males vary during, and shortly after, metamorphosis, and these were found to lie between 2:1 and 5:1. A normal ratio is however achieved by the fall. According to the statistical investigations of Pflüger (1882) and R. Hertwig (1905 - 1907), these strains may be considered as being well differentiated ones in similarity to those known to exist in the North (Königsberg - Kaliningrad) and in the Central European highlands. Here in Dorpat, too, the ratios vary greatly from finding- /387 place to finding-place and from year to year. I, for instance, have found females only during metamorphosis on the southern slopes of the hills surrounding the Embach river, a region where frogs spawn much earlier. Regarding such findings, one may consider the influence exerted by temperature on the same strain. According to Witschi (1914) low temperatures promote the formation of males. I may furthermore, mention that the sex of the animals can be determined in these local strains very early, i. e., well before metamorphosis. During

* Numbers in the margin indicate pagination in the foreign Text

** Cf. the preceding communication by Lipschütz.

metamorphosis the ovaries exhibit their characteristic structure with clusters of well-developed eggs, some of which having already advanced to the pachytene stage. In the spring of 1921, during which time the experiments described in this paper were carried out, frogs spawned very early in the season, i. e., during the first days of April on the north-eastern slopes of the hills. Toward the end of spawning-time, on April 10th, a mating couple of frogs was captured in the field and transported to the institute, where the animals were separated for approximately 72 hours; one part of the eggs was then fertilized in the usual manner. The other part of the eggs was kept for 24 hours in a moist chamber and these eggs were fertilized with the sperm of a different male. The eggs employed had, thus, achieved a high degree of uterine overmaturity, a fact demonstrated by the high mortality exhibited by the eggs fertilized after 96 hours. After fertilization almost all the black poles migrated to the top of the eggs demonstrating that almost all eggs were viable and that a spermatozoon had, in fact, penetrated into the egg. A great number of eggs died during the early stages of vitelline development. On April 26th and 27th, respectively, 56 tadpoles were hatched from the 76 eggs of the 72-hour-series, and just 70 tadpoles from the 280 eggs of the 96-hour-series. These findings would indicate that it is almost certain that the time interval employed in this instance represents the extreme during which fertilization can be achieved.

On May 2nd, the tadpoles, which by that time had utilized their gelatine sacks, were transferred into four glazed clay vessels each containing approximately 120 ml of water, which was changed practically every day. The water used was tapwater, and it contained great concentrations of calcium carbonate. Later on the amount of water added was increased until it finally amounted to 550 ml.

The first vessel (72-h-♀) contained 28 tadpoles of the 72-hour-series, and these were fed exclusively with ovaries obtained from adult frogs. The ovaries were always ground in a mortar before feeding.

The second series (72-h) contained 28 tadpoles of the same series, and these animals were fed with frog liver, kidney, spleen and, occasionally, testes.

The third vessel (96-h-♀) contained 35 tadpoles of the 96-hour-series; these were fed with ovaries like the 72-h-♀-series.

The fourth vessel (96-h) contained 35 tadpoles of the 96-hour-series, and these were fed like the 72-h-series.

During the first two weeks, filamentous algae were added to the water, but because of the inherent danger of a parasitic infection, this algae addition was later on discontinued. Most of the animals of the second 72-h-series died. Later on mortality was less.

/388

The vessels were kept at a window of the institute facing north. The temperature varied between 18° and 25°C.

On June 7th, the differences in size of the tadpoles were very marked. The largest tadpole of the first 72-h-♀-series exhibited a weight of 0.35 g, and that

of the 96-h-series one of only 0.17 g. It should be noted that feed was in all series offered in excess. The hindlimbs of animals belonging to the first 72-h-♀-series were markedly larger. The animals of the second 72-h-series and the 96-h-series were between those of the two groups just mentioned. The animals were sacrificed on the day when their forelegs appeared; they were weighed before sacrifice and then preserved in a 10% formaldehyde solution. The preserved animals were labelled, and they could easily be distinguished during histologic examination. Apart from the animals of the second 72-h-series, of which approximately three quarters died, a total of 74 tadpoles belonging to the three other series (72-h-♀, 96-h-♀ and 96-h) was examined; 24 tadpoles had died.

Results

The results are summarized in the Table.

TABLE

Water	72h♀	72h	96h	96h♀
Designed for research	28	28	35	35
Examined microscopically.....	24	8	27	23
Age in days (average).....	72.5	79.0	78.0	109.0
Weight in grams (average)	0.290	0.210	0.294	0.175
Number of females	13	6	12	18
Number of hybrids ♀	8	2	10	(1)
Number of males	3	—	5	4
Size of the eggs and cores in μ	30	23	28.6	57
(average)				
Size of the ovaries in μ (average)	19.4	21	18.7	38.3
Length	1280	1040	1100	1154
Width	285	270	273	329

The table demonstrates the following findings:

1. Feeding of ovaries did not produce a shift in the numerical sex ratio: the numbers of females in the 72-h- and the 96-h-series are not smaller than those in the corresponding 72-h-♀ and 96-h-♀-series.

2. Feeding of ovaries, however, did accelerate metamorphosis. Comparison of the 96-h-♀- and 96-h-series in particular makes this fact evident. In the 96-h-series, 9 tadpoles did not undergo metamorphosis; these animals had to be killed since they were close to dying between the 120th and 144th (!) day after fertilization. The greater weight of the animals fed ovaries corresponds to the weight exhibited by animals found in the field.

/389

3. Late fertilization caused a delay in metamorphosis. Comparison of the 72-h and the 96-h-series and that of the 72-h-♀- and 96-h-♀-series demonstrate this point.

4. In the frog, development of the ovaries and their product in independent from that of the body. Development of the former is a function of time. The 72-h-♀, 72-h- and 96-h-♀-series, thus, exhibited similar values for size at the moment of metamorphosis; the same also held true for the size of the eggs and the thickness of the ovaries. The 96-h-series differed in these respects greatly from the three former.

5. Late fertilization did not produce 100% of males as one would have expected in accordance with the results obtained by Hertwig and others. The number of ascertained males corresponded approximately to that found by me in the fields around Dorpat: The ratio of females to males was 5:1.

6. The three first series exhibited a greater number of intersex animals. In the fourth series, intersex forms were not present at all. The case shown as ♀ in this series represents a lateral hermaphrodite.

How then can one reconcile the findings obtained by Hertwig and his students—who after extensive uterine overmaturity of eggs always found 100% of males to develop—with those just described. The findings obtained may, perhaps, be more easily understood when one assumes that late fertilization brings about a favored development of males only through a mechanism in which the female factors always present (the primordium, of course, is of hermaphroditic nature) are inhibited at some point early in development. In the later course of development, these factors again become effective and assume their original preponderance over the male factors. The newly developing ovarian tissue would replace the testicular tissue, which process would represent a sex transformation, a process known to occur in the field in the opposite direction only (cf. further below). It is conceivable that R. Hertwig and other investigators have determined the sex of the gonads at a very early stage of development only. Development of the gonads, however, frequently is retarded, and R. Hertwig has mentioned cases in which gonads were still indifferent in the second year.

Do we have evidence pointing to the actual occurrence of sex transformation in the course of tadpole development? Since it is not possible to examine the sex of the same specimen repeatedly in the course of development, one is forced to employ the methods of Schmidt-Marcel (1908). The latter author used a table showing the numerical sex ratios found in froglets of his large cultures in the 1st, 2nd, 3rd etc. months after metamorphosis. His table showed that initially there were 85% of females and 15% of males present, and that the number of males gradually increased at the expense of the females, while intersex forms simultaneously appeared. Normal numerical ratios were finally established and the intersex types disappeared again. During the microscopic examination of the intersex gonads, Schmidt-Marcel was able to demonstrate the occurrence of various transitional stages between females and males. Transformation of one sex into the other one was thus demonstrated.

Similar evidence may be found in my table, with the difference, however, being that the direction of transformation is a different one. The first stage,

where a total of 100% of males should have been present due to late fertilization cannot be demonstrated at all. My table exhibits two time interval values viz. the 72-h-ii, 72-h and 96-h-♀-series 72-78 days, and the 96-h-series 109 days at an average. One can see that the number of females increased at the expense of the intersex forms. The number of ascertained males—the gonads of which, by the way, are much smaller than those of intersex and female animals (approximately 0.8/0.2 mm)—was approximately the same in all series. The gonads were spindle-shaped and they contained germ cells exhibiting resting nuclei only. Structures looking like clusters of eggs were not found. The parenchyma consisted of a large number of ampullar anlagen, which were interconnected centrally; they were not arranged in series. Cavities were frequently found. Ovaries of the corresponding developmental stage always exhibited clusters of eggs containing relatively highly developed eggs; pseudo-reduction stages and young eggs were always found. The microscopic picture of the testes corresponded largely to those found by Kuschakewitsch (1910) in *Rana esculenta* in his Fig. 24 and by Witschi (1914) in *Rana temporaria* in his Figs. 30-37.

The sex of the females shown in the column denoted "females" was not in doubt. Ovarial pockets lined by a single or a double layer of endothelial cells containing micronuclei were found in great numbers in this particular tissue. These pockets were compressed by the egg clusters to a greater or lesser extent. The size of the eggs varied with the age of the gonad, and it was largest in the oldest ones, and this even when larval gonads were examined; eggs exhibiting a size of 90μ with nuclei measuring 55μ were found in the 96-h-series. Some coagulum matter was occasionally found in the ovarian pockets, and the possible significance of this finding will be discussed further below.

It should be mentioned that occasionally ovaries were found which did not exhibit clusters of young eggs with pseudo-reduction stages. Instead of these, I found a loose, vacuolized mesenchymal tissue filling the spaces between the larger eggs (50μ). The eggs did not touch each other, which is in contrast to findings obtained in normal ovaries. As may be seen in the table, the ovaries of the 96-h-series contained the largest eggs. /391

About the intersex animals, the gonads of which exhibit reproductive tissue of both sexes, I have to report the following: At first glance, the smaller gonads appear to be testes. They remind one of the testes of *Rana esculenta* of the IIb Series shown in Fig. 95 of Kuschakewitsch's paper. The microscopic examination showed ampullar anlagen arranged in series. The multilayered wall of these ampullar anlagen exhibited one more or less large cavity. Figures 2 and 3 represent longitudinal sections. Arrangement in series and the cavity in the ampullae, which derive from the genital funicle, is characteristic for an ovarian anlage; the multilayered wall of the sacks, however, is characteristic for a testicular anlage. At the border of the gonad, interspersed between the ampullae, one may see a number of clusters of eggs more or less developed. Figure 1, for instance, shows a proximal ampullar anlage of a 70-day-old gonad of the 72-h-♀-series. One gonad exhibits approximately six such ampullae. The proximal ampulla exhibits a cavity, which form of structure is not exhibited by the other, distal, much smaller ampullae. This cavity contains a serous coagulum matter, the particles of which stain strongly. In the right upper corner one may see a circular concentration of cells, which apparently represents an egg cluster at a very early stage of development. It contained gonocytes with large nuclei. /392

Figure 2 shows parts of an older gonad (of the 96-h-♀-series on the 75th day). The ampullar cavities are larger and they contain some coagulum matter. In relation to the cavity, the wall, although multilayered, is much thinner than the ampullar wall shown in Fig. 1. The arrangement of the cells containing small nuclei in the interior of the ampullar anlage has an endothelial character. The clusters of eggs (upper right and left) are larger than those shown in Fig. 1, and they can easily be recognized as such.



Figure 1.
(72-h-f-♀-series)

Figure 3 (72-h-♀-series on the 76th day) exhibits a picture frequently encountered, which may throw some light on the developmental mechanism of the ampullar cavities. This picture shows relatively thin-walled ampullae containing in the interior some degenerating poorly staining cellular material, which apparently is derived from the walls. The ampullar wall exhibits a rather irregular appearance due to the fact that the rapidly growing egg clusters are pushing outward.

/393

Figure 4 shows a longitudinal section of a gonad of the 96-h-♀-series (78 days old). The serial arrangement of the testicular ampullae can be clearly seen. Some coagulum material with pyknotic nuclei can be seen in the cavity. Relatively large egg clusters containing rather large eggs are present.

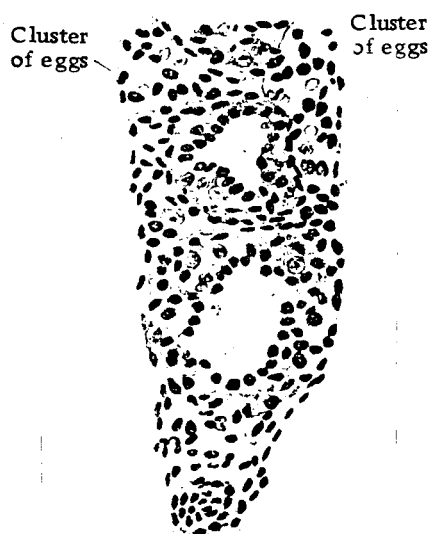


Figure 2. (96-h-♀-series).

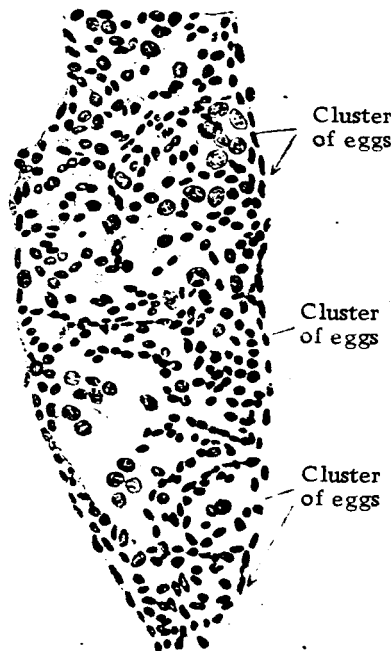


Figure 3. (72-h-♀-series).

Figure 5 shows the distal part of a gonad of the 96-h-ii-series (on the 85th day). The cavities exhibited by the ampullae arranged in series are very large, and they contain coagulum material. The walls are lined by a double or triple layer of cells only. The nuclei are closely packed. Between the individual ampullae one can see a number of egg clusters as well as a few large single eggs close to the surface. This gonad differs from a true ovary only by the multilayered wall exhibited by the ovarian pockets; that we are in fact dealing with such pockets can hardly be doubted. Figures 1-5 exhibit a number of transitional forms between—actually not truly normal—testicular tissue (Kuschakewitsch' normal type IIb) and ovarian tissue. That the development proceeds in this direction and not in the other one is demonstrated by the fact that the intersex forms disappeared in the 96-h-series and that these were replaced by females and not by males, as well as by the fact that the gonads were larger. The genital spaces simultaneously increased in size. The coagulum material in the secondary genital spaces of the ovary—in particular in the 96-h-series—speaks for a masculine past. It should be noted that the only hermaphroditic form found in the 96-h-series was a lateral one, in which one gonad exhibited a normal testicular structure with no serial ampullar

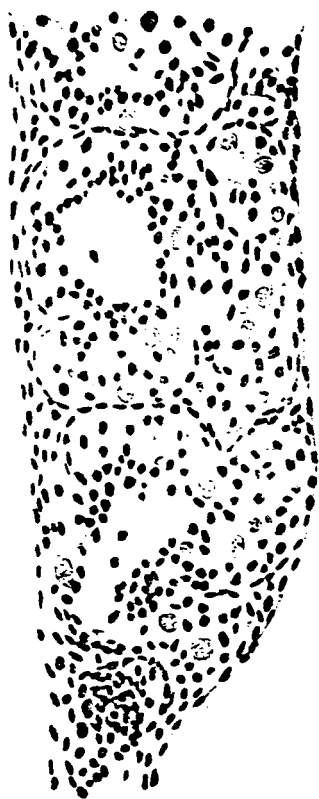


Figure 4. (96-h-♀-series).

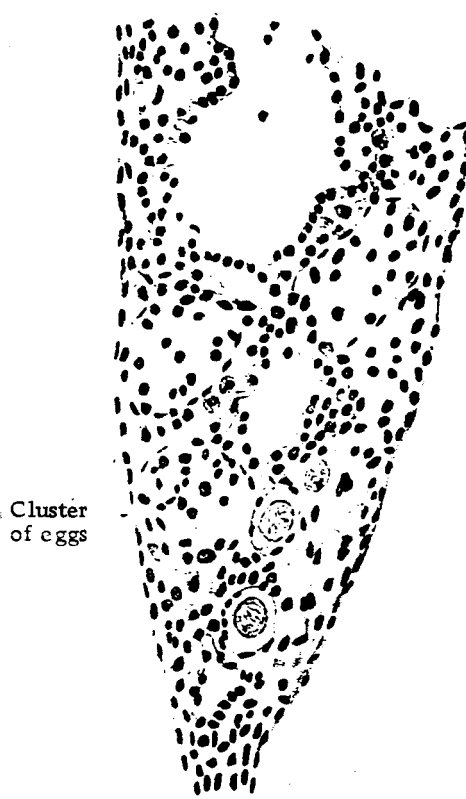


Figure 5. (96-h-♀-series).

anlagen present, while the other gonad exhibited an ovarian structure with eggs measuring approximately 40 μ in diameter present; early egg clusters were not found.

The findings reported demonstrate that 100% of males do not necessarily develop in all instances from highly overmature uterine *Rana fusca* eggs, and that sex transformation in the frog in the course of development may also proceed in the direction from male to female. A complete ruination of the female-sex-determining factor should, thus, not be supposed to take place in the overmature frog egg.

A deviation from the normal of the numerical sex ratio following overmaturity of the eggs is caused not by a direct influence exerted on the mechanism of sex distribution (Goldschmidt 1921, p. 224) but rather by a temporary inhibition of the female-sex-determining factor.

The question arises now whether a part of the females of the 96-h-series would have been re-transformed into males if they had stayed alive for a longer period of time, in this way leading to the re-establishment of the normal sex ratio (1:1).

/394

Our investigations will be continued in the course of this year with the aid of experimental material considerably larger in numbers.

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